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Composition, abundance and diversity of immature Odonata (INSECTA) insects living in stretches of middle Araguari River, Amapá State, Amazonian Region, Brazil

Odonata order, popularly known as dragonflies, comprises insects belonging to group Palaeoptera and presents hemimetabolous development encompassing three life cycle stages: eggs, larvae and adults. Odonata order has three suborders, namely: Anisoptera, Zygoptera and Anisozygoptera - the last one is exclusively distributed in Asia. The aim of the current study is to investigate the composition, abundance and diversity of Odonata larvae in middle Araguari River (Amapá, Brazil) by evaluating space-seasonal fluctuations and their association with different substrates. Eight collection sites were set along the river. Quarterly samplings were performed within an annual cycle (April/2018 to April/2019) by positioning a trawl "D" net (Rapiché) on the riverbed and scraping the substrate to collect 2L of sample. Odonata richness, abundance and diversity indices were evaluated. Data were statistically treated based on simple descriptive analysis, means, medians and standard deviations, Kruskal-Wallis test (nonparametric data) and ANOVA (parametric data) in the R-statistics Software. Two hundred and ninety-seven (297) individuals were identified; Libellulidae was the most abundant family (80.13%). Diversity and equitability have shown variations between sampling sites (p<0.05) and collection campaigns (p<0.05). Only abundance was influenced by categorical variables such as downstream/upstream river and substrate type. Higher taxonomic resolution, in combination to environmental variables, could help establishing ecosystem relationships that were not identified in the current study.

Keywords: Odonatofauna; Libellulidae; Eastern Amazon.

Composição, abundância e diversidade de imaturos de Odonata (INSECTA) em trechos do médio Rio Araguari, Amapá, Amazônia, Brasil

A ordem de Odonata, popularmente, conhecida como libélulas, compreende insetos pertencentes ao grupo Palaeoptera e apresenta desenvolvimento hemimetabólito que abrange três estágios do ciclo de vida: ovos, larvas e adultos. A ordem odonata possui três subordens: Anisoptera, Zygoptera e Anisozygoptera - a última é distribuída exclusivamente na Ásia. O objetivo do presente estudo é investigar a composição, abundância e diversidade de larvas de Odonata no médio rio Araguari (Amapá), avaliando flutuações espaço-sazonais e sua associação com diferentes substratos. Oito sítios de coleta foram distribuídos ao longo do rio. As amostras trimestrais foram realizadas dentro de um ciclo anual (abril/2018 a abril/abril/2019), posicionando uma rede de arrasto "D" (Rapiché) no leito do rio e raspando o substrato para a coleta de 2L de amostra. Foram avaliados os índices de riqueza, abundância e diversidade de Odonatas. Os dados foram tratados, estatisticamente, com base em análises descritivas simples, médias, medianas e desvios padrão, teste de Kruskal-Wallis (dados não paramétricos) e ANOVA (dados paramétricos) no Software R-statistics. Foram identificados 297 indivíduos, onde Libellulidae foi a família mais abundante (80,13%). A diversidade e equitabilidade mostraram variações entre os locais de amostragem (p<0,05) e campanhas de coleta (p<0,05). Apenas a abundância foi influenciada por variáveis categóricas, como jusante/montante do rio e tipo de substrato. Uma maior resolução taxonômica, em combinação com variáveis ambientais, poderia ajudar a estabelecer relações ecossistêmicas que não foram identificadas no presente estudo.

Palavras-chave: Odonatofauna; Libellulidae; Amazônia Oriental.

Topic: **Fundamentos de Biodiversidade**

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INTRODUCTION

Benthic macroinvertebrates form a group of widely tested organisms that have been used as biological indicators over time. They are used for it because of their ability to live throughout the aquatic spectrum, often associated with unconsolidated substrates composed of stones, plants and animals or buried in mud or sand sediments at the bottom of aquatic environments, for at least one stage of their lives, and it shapes their benthic habit (ZARDO et al., 2013; BARONI et al., 2015; BARBOSA et al., 2016). This group comprises a variety of organisms, such as Insecta, Crustaceans, Oligochains, Gastropods, Bivalves, among others, mainly in freshwater environments (BUSS et al., 2003; TUNDISI et al., 2008; CETESB, 2012; CHAGAS et al., 2017).

Order Odonata, popularly known as dragonflies and washers, comprises organisms that stand out among macroinvertebrates used to monitor water bodies. Members of this order, whose larvae present sedentary habit, can fully occupy aquatic systems, in association with some substrate types. Besides, their larval stage can last from weeks to years and they also have specific ability to tolerate environmental disturbances (CARLE, 1979; NEISS et al., 2014).

Dragonfly fauna distribution in Brazil remains poorly known, since only 29% of the Brazilian territory presents data about Odonata richness and only approximately 3.5% of the total Brazilian territory was inventoried, so far (MARCO JÚNIOR et al., 2005). However, many experts has emerged in the country in recent years, a fact that has significantly contributed to improve taxonomic knowledge about this order, which encompasses approximately 10% of studies on aquatic insects (MELO, 2014; NESSIMIAN et al., 2014)

The Brazilian Amazon itself is a challenge to taxonomic knowledge about its biodiversity, not only to order Odonata, but also to other macroinvertebrates. Because the Amazon is a neotropical region, it is assumed that there may be individuals yet to be described, which would lead to a richer, and yet unknown, biodiversity (NESSIMIAN et al., 2014). Thus, Amapá and Acre are the Amazon states presenting the smallest number of studies about Odonata description, distribution, and ecology. According to Pinto (2016), South America is the "last frontier to be unveiled" when it comes to dragonfly fauna; thus, it is necessary investigating the composition of Odonata families living in different ecosystems in this region.

Therefore, the aims of the current study were to investigate the composition, abundance, and diversity of order Odonata in middle Araguari River region, as well as to evaluate space-seasonal variations and the association between Odonata fauna and substrate type to help improving knowledge about this topic, which remains incipient in the region.

MATERIALS AND METHODS

Study site

Araguari River basin is in the Amazon hydrographic region, Northernmost Brazil. It lies between parallels 00º 25' N and 02º 28' N and meridians 49º 53' W and 53º 02' W. Its headwater is located South to Lombarda mountain range in Tumucumaque Mountain National Park, and flows approximately 498 km into its mouth, which flows right into the Atlantic Ocean – it is the largest/exclusive hydrographic basin in the State (SANTOS et al., 2018; SILVA et al., 2018).

Araguari River basin can be divided into three stretches with different hydrological features: the upper course, which is 132-km long, lies between the confluence of Tajuí River and Porto Grande County; the middle course, which is 161-km long, lies between Porto Grande and Ferreira Gomes counties and is featured by high water speed and turbulence; and the low course, which is 205-km long, runs from Ferreira Gomes County to its mouth (SANTOS et al., 2018; SILVA et al., 2018). The current research has selected the middle Araguari River as study site.

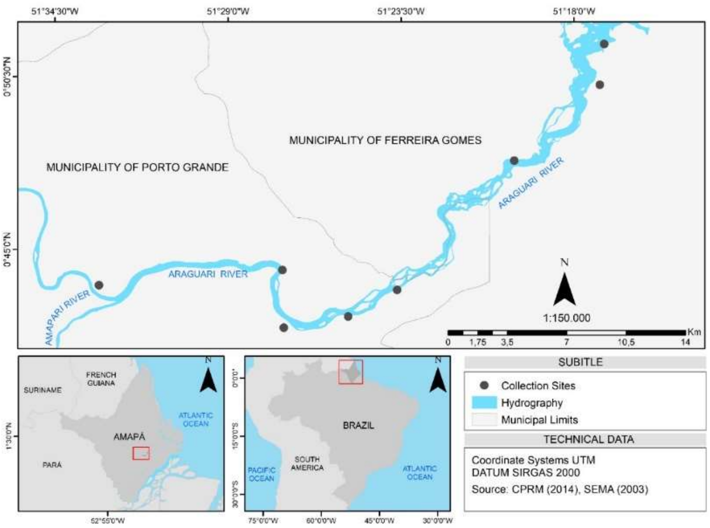

Figure 1: Location of the middle Araguari River course.

Sampling sites and periodicity

Eight collection sites were distributed along middle Araguari River, based on features of associated substrates (Table 1). Each site was segmented in triplicate, called "A", "E" and "J", to achieve sample sufficiency in the investigated environment. Collections were carried out on a quarterly basis throughout a one-year cycle (April/2018 to April/2019).

Table 1: Geographical coordinates and description of sampling sites

Sampling Sites	Geographic Coordinates	Substrate type
CC1	0º 51'327" N 51º 17'128" W	**Sand**
CC2	0º 50'842" N 51º 17'276" W	Stone
CC3	0º 47'495" N 51º 19'512" W	**Decaying organic matter**
CC4	0º 43'290" N 51º 24'16" W	Decaying organic matter
CC5	0º 43'252" N 51º 25'478" W	**Vegetation and stone**
CC6	0º 48'252" N 51º 21'678" W	Vegetation and aquatic macrophytes
CC7	0º 44'527" N 51º 24'292" W	**Decaying organic matter and aquatic macrophytes**
CC8	0º 44'133" N 51º 33'534" W	Sand

Collection and sampling methods

A trawl "D" net (Rapiché) was placed on the riverbed to scrap the substrate and collect 2L of sample. Next, samples were fixed and preserved in ethyl alcohol at 92°; labels were used to identify them and to make the subsequent screening and analysis, based on laboratory methodology suggested by Bicudo et al. (2004), easier.

Collected material was screened at the Invertebrate Zoology Laboratory (LABINVERT) of Federal University of Amapá, Macapá City. The separation of collected organisms and associated substrates was carried out with the aid of two overlapping sieves (2 mm and 0.5 mm mesh). The identification and separation of immature organisms were carried out with the aid of translucent trays, supporting lights, tweezers and dropper (CARVALHO, 2007). Captured individuals were stored in *vials* filled with 70% ethyl alcohol.

Organisms were identified under stereoscope microscope (magnifying glass), based on taxonomic identification keys (MCCAFFERTY, 1983). Taxonomic resolution was used at family level in the current study.

Ecological indices

The following ecological indices were evaluated in the current study: richness, abundance, and diversity. Richness is understood as the index representing the total number of taxa found in each area or sampling unit. It helps featuring specific diversities in studies conducted in this field (SENNA et al., 1999).

Abundance is the total number of individuals identified in each area, location or sampling unit. Relative abundance (S*pi*), in its turn, refers to the number of individuals in each taxon in comparison to the total number of organisms identified per area, place or sampling unit (GOMES et al., 2004). It can be calculated through the following formula:

$$Spi = n.(\frac{100}{N})$$

Wherein:
n = total number of identified organisms.
N = total number of organisms identified per taxonomic family.

Specific diversity is defined as the number of taxa identified in each sample, area unit, water volume, certain number of individuals, among others, in each time unit (SENNA et al., 1999). Shannon-Weaver index was used in the current study to identify a specific diversity (H') (SHANNON et al., 1963), because it is lesser dependent on sample size and widely used in ecological studies. This index is defined by the following equation:

$$H' = -\ \Sigma\ (pi\ x\ log\ pi)$$

Wherein:
pi = Ni/N.
Ni = species density.
N = total sample density.

Equability index (J) ranges from interval 0 to 1; thus, the closer to 1, the greater the equitability, which points out homogeneous distribution of taxa abundance. Equitability can be defined through the equation below:

$$J = \frac{H'}{Hmax}$$

H_{max}= ln (S).
J = Equability index
S = total number of sampled species.
H' = Shannon-Weaver diversity index.

Data analysis

Data were tabulated in Excel spreadsheets and imported to the R-statistics and Past 3.0 software, which were used to carry out statistical tests and generate graphs.

Descriptive statistics was performed in R-statistics software to identify the means, medians, standard deviations and confidence levels (95%) of tabulated data (composition and abundance). Composition and abundance data did not present normal distribution (Shapiro-Wilk test), which led to the adoption of non-parametric statistical tests. However, data on abundance and type of substrate reached normality, which enabled subjecting it to statistical parametric analysis.

Variations in medians and means were carried out through Kruskal-Wallis and One-Way ANOVA tests, respectively, based on the following variables: a) sampling sites, b) collection campaigns, c) substrate type and d) Downstream/Upstream river. Tests were performed in R-statistics software; statistical significance was set at α=0.05.

Species richness estimators (Chao 2, Jackknife 1, Jackknife 2, and Bootstrap) and rarefaction curve were used to analyze sample composition and sufficiency in the study site, in the Past 3.0 Software. This software was also used to calculate species diversity (Shannon-Winner) and equitability (Pielou index). Significant differences (α=0.05) in diversity and equitability between sites and sampling periods were checked through one-sample t-test, in R-statistics Software; statistical significance was set at α=0.05.

RESULTS

The total number of 297 individuals belonging to Order Odonata were collected: 273 belonged to suborder Anisoptera and 24, to suborder Zygoptera. Individuals were distributed in five families: Aeshnidae (n=1), Coenagrionidae (n=20), Gomphidae (n=34), Libellulidae (n=238) and Megapodagrionidae (n=4). Libellulidae was the most representative family, since it presented relative abundance of 80.13%. It was followed by families Gomphidae (11.45%) and Coenagrionidae (6.73%) (Figure 2).

Randomized rarefaction curve (Figure 3) generated for species richness in the study site was stable. However, the curve did not reach the asymptote, it presented small trend to increase, which suggested that as new samples increased in the study site, there may have been increase in specific richness.

Although the rarefaction curve did not get stable, richness estimators (Chao 2, Jackknife 1, Jackknife 2, and Bootstrap) recorded values similar to those observed for the study site (Table 2), which ranged from 5.72 to 8.85.

Species richness did not show significant variation among sampling sites (H= 3.7282, p= 0.810), collection campaigns (H= 3.8379, p= 0.4284), downstream/upstream river (H= 1.3323, p= 0.2484) or substrate type (H= 3.0685, p= 0.8002).

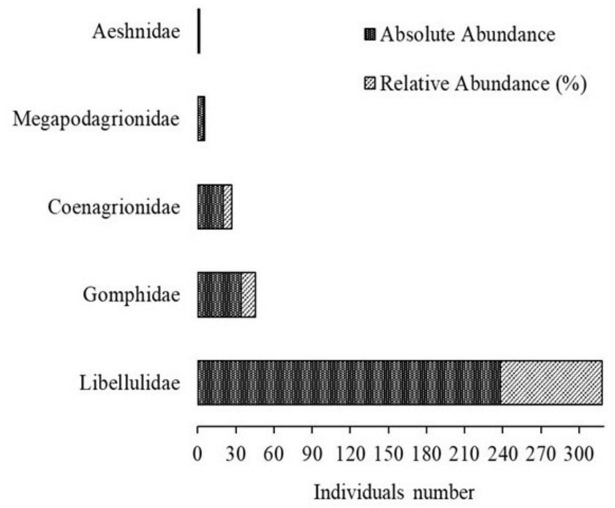

Figure 2: Absolute and relative abundance of taxonomic families of Odonata identified for study area.

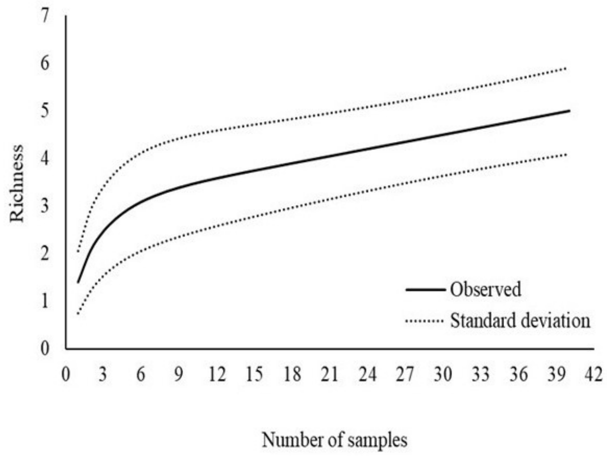

Figure 3: Odonata taxa accumulation curve and standard deviation (95% confidence interval) collected along the stretch of the medium Araguari river.

Table 2: Values of richness estimates for study area.

Estimators	Richness	Standard deviation (±)
Observed	5	-
Chao 2:	5.97	0.851704
Jackknife 1	6.95	1.17182
Jackknife 2	8.85	1.88897
Bootstrap	5.72	0.851514

Species abundance did not show significant variations between sampling sites (H= 10.414, p= 0.1663) and collection campaigns (H = 3.4492, p= 0.4856) (Figure 4). However, there was significant variation between variables "abundance" and "downstream/upstream river" (H=3.8284, p≤0.05) - the highest values were associated with upstream points in the river (N=11.30) (Figure 5). Similarly, there was significant variation in species abundance depending on substrate type (F = 2.881, p≤ 0.02) - the highest values were observed for Sand (n=18.1) and Vegetation and Stone (n=16.6) substrates (Figure 5).

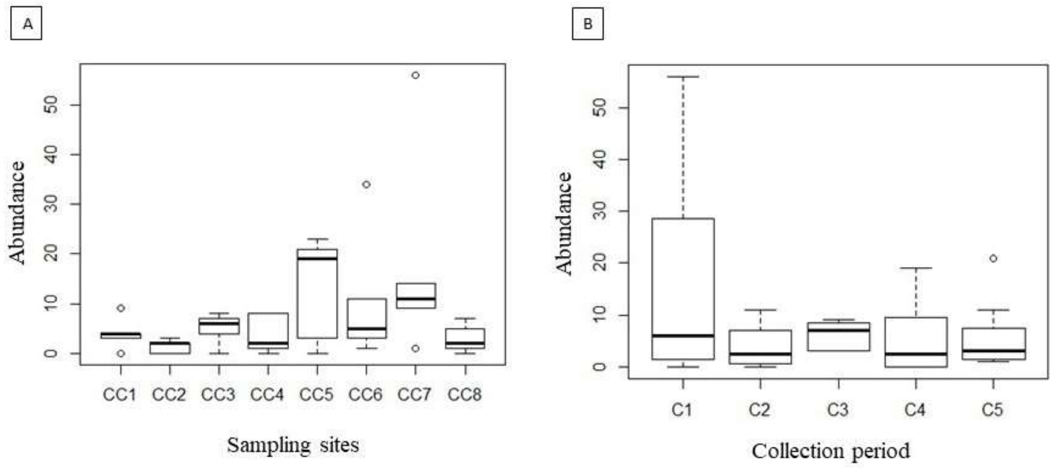

Figure 4: Variation of the abundance of Odonata larvae between sampling sites (A) and collection period (B).

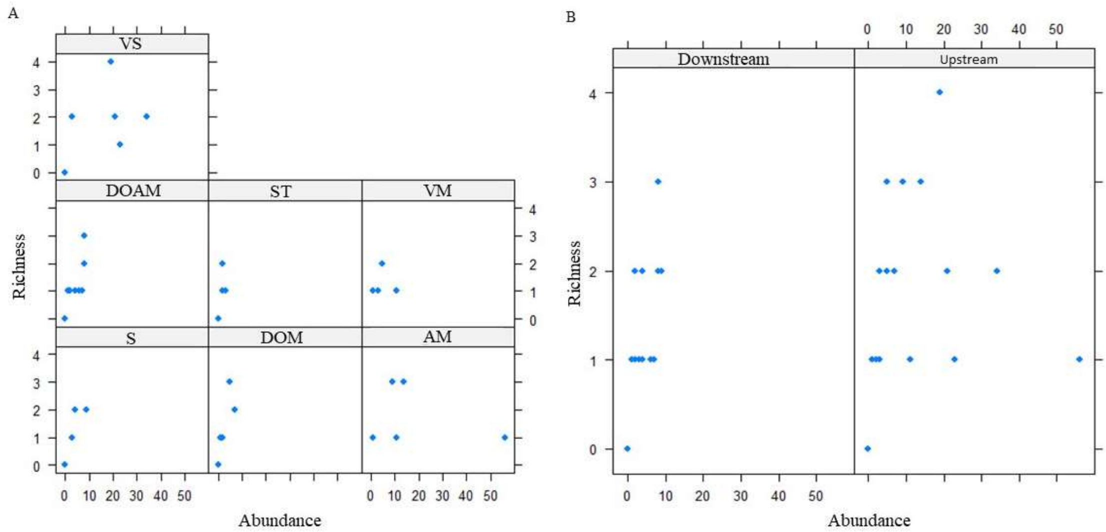

Figure 5: Variation of the abundance of Odonata larvae in relation to the type of substrate (A) and downstream/upstream river (B). S: Sand; DOM: Decaying organic matter; AM: aquatic macrophytes; DOAM: Decaying organic matter and aquatic macrophytes; ST: Stone: VM: vegetation and aquatic macrophytes; VS: vegetation and stone.

Shannon diversity has shown significant variation between sampling sites (t = 32.221, df = 7, p-value = 0.0000000007173) and collection campaigns (t = 12.308, df = 4, p-value = 0.0002504). The highest diversity value was observed for site CC3 (H'=1.35) and for C3 campaign (H'=1.98), whereas the lowest value was recorded for site CC6 (H'=1.07) and for the C4 campaign (H'=1.22) (Table 3).

Similarly, equitability has shown significant differences between sampling sites (t = 19.955, df = 7, p-value = 0.0000001985) and collection campaigns (t = 19.347, df = 4, p-value = 0.00004208). The highest values were observed for site CC2 (J=0.98) and for C3 campaign (J=0.95), whereas the lowest values were observed for site CC6 (J=0.66) and for C1 campaign (J=0.72) (Table 3).

Table 3: Shannon diversity index and Pielou equitability index based on sampling site and collection campaigns.

	Sampling site							
	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8
Shannon	1.288	1.079	1.357	1.12	1.231	1.07	1.121	1.171
Pielou	0.9289	0.9821	0.9787	0.8082	0.8878	0.6649	0.6962	0.8447

	Collection campaigns				
	C1	C2	C3	C4	C5
Shannon	1.407	1.549	1.987	1.227	1.564
Pielou	0.7229	0.8643	0.9557	0.8848	0.7521

DISCUSSION

Studies with Odonata larvae are abundant in the literature, which mainly presents fauna surveys and analyses of aquatic biomonitoring of these organisms. However, such studies remain scarce or are almost non-existent in the Amazon region, depending on the microregion.

The present study has identified the total number of 297 individuals belonging to Order Odonata, which were distributed into five families (Aeshnidae, Corduliidae, Gomphidae, Libellulidae and Megapodagrionidae) and two suborders (Anisoptera and Zygoptera). Libellulidae was the most abundant family, which accounted for more than 80% of the total sample. It was followed by families Gomphidae

(11.45%) and Corduliidae (6.73%). This pattern is often found in neotropical regions, where these three families often prevail. Similar findings were described in other studies, such as the ones conducted by Hanauer et al. (2014) and Soares et al. (2015). However, no other study has observed such a family-concentrated distribution like the ones observed in the current study.

Taxa belonging to family Libellulidae are the most abundant within order Anisoptera; they account for 48% of neotropical species and present high species richness (COSTA et al., 2012). Similar to its adult individuals, larvae belonging to this family present wide variety of shapes; they often have robust bodies and strong legs (NEISS et al., 2014). Some studies have shown their potential to be used as degraded environment indicators, since some species are capable of occupying affected freshwater environments lacking marginal vegetation, as well as presenting silted margins, significant pollutant concentrations and pH changes (CORTEZZI et al., 2009; CORBI et al., 2011; DALZOCHIO et al., 2011). In addition, these organisms can colonize different environment types, such as temporary (puddles, swamps, and wetlands) and lotic environments (NEISS et al., 2014). When it comes to middle Araguari River, these organisms appear to have presented good adaptation profile, since they were found throughout the sampling and collection sites.

In addition to family Libellulidae, two other families - Gomphidae and Coenagrionidae - were often observed at the study site throughout the year; they were considered likely environmental bioindicators (FERREIRA-PERUQUETTI et al., 2003). Family Gomphidae is described as the second richest family among Anisopteras (NESSIMIAN et al., 2014).

Larvae belonging to family Coenagrionidae in middle Araguari River are often found in rivers, streams, lakes, puddles, among others. They live in different substrate types, mainly in leaf litter in backwater or current areas (NEISS et al., 2014). Substrates associated with families Gomphidae and Coenagrionidae were observed in the current study. This finding has emphasized the constancy of these taxa during the sampling periods.

No categorical variable has influenced the composition of families cataloged throughout the research. Only abundance was significantly influenced by variables "downstream/upstream river" and "substrate type". Accordingly, Camargo et al. (2019) has emphasized that human presence leads to environmental impacts capable of destabilizing the trophic network and of reducing local diversity, since the most adapted and competitive species are often the prevalent ones. Variation in sampling site position (upstream and downstream) corroborates the substrate type influence, since substrates associated with higher species abundance were concentrated upstream Araguari River.

Overall, Amazonian insects prefer certain substrate types given the multiple functions they may have in the environment, such as providing shelter, help transporting these organisms, protecting them from predators and, in some cases, working as food (ASSIS et al., 2004; BARBOLA et al., 2011; TURRA et al., 2018). The number of Odonata families living in aquatic environments often decreases when destructuring processes affect the substrate (TURRA et al., 2018).

It is possible observing two immature Odonata specialization types towards substrates occupied by them (ASSIS et al., 2004). One group lives buried in or covered by fine sediment (fossorial and reptants), whereas the other is found in larger and more stable substrates (grabbers and climbers). Both groups were observed in middle Araguari River, since their highest abundance was associated with substrates featured by the presence of fine sediments and stones.

The highest Shannon (H') diversity values were observed for site CC3, and it suggested better distribution of organisms, mainly of Libellulidae and Gomphidae. This very same site has shown greater equitability (J), as well as site CC2. The highest values recorded for attributes such as H' and J have emphasized the good integrity condition of these habitats, mainly riverbed stability, because they presented low current flow and presence of marginal vegetation. This feature likely helps reducing eventual disturbances, mainly during heavy rainfall events. These factors contribute to the abundance of other invertebrate types, which end up enabling the successful establishment of immature Odonata individuals who use them as food (RIOS et al., 2006).

Organism diversity has also shown significant variation between collection periods - the highest values were recorded for campaign C3. The same pattern was observed for equitability. This campaign took place in October, which is the driest period of the year in Amapá State and, consequently, presents the lowest rainfall rates in the region (SOUZA et al., 2010).

Thus, seasonality has strong influence on immature Odonata development, as reported in other studies (CORBET, 1980). It happens because the dry period acts as ecological filter and enables thermal amplitude variations that lead to lower food availability. This process favors the survival of smaller individuals because they are lesser demanding in their diet (Batista, 2010). Therefore, it is possible inferring that seasonality in the region affects the diversity (richness and equitability) of immature Libellulidae, Gomphidae and Coenagrionidae individuals during the lesser rainy periods in middle Araguari River.

CONCLUSIONS

This is the first study carried out with Odonata larvae in the middle course of Araguari River. Studies such as the current one are extremely important for preliminary knowledge development. Moreover, they enable performing other studies not only based on the environmental perspective, but also on the social and economic ones.

The taxonomic composition of immature Odonata comprised 297 organisms; Libellulidae was the prevalent family since it accounted for more than 80% of the entire inventory. Thus, the middle stretch of Araguari River does not present rich taxonomic composition in terms of families, since it presented three of the four Anisoptera families and two of the ten Zygoptera families described in Brazil, which is a low pattern if one takes into account its neotropical region feature.

Abundance was the only index influenced by categorical variables such as downstream/upstream river and substrate type. However, this variation was mostly associated with the influence of substrate type, since substrates presenting greater Odonata abundance were in the upstream portion of Porto Grande

County.

Diversity and equitability were also influenced by seasonality. This outcome enabled inferring the influence of the lesser rainy period on taxa distribution. The hydrodynamics of the river changes during this time, since it presents lower water speed and flow, as well as enables Odonata fauna development.

Finally, further research should be developed to achieve higher taxonomic resolution based on a larger number of measured variables, such as those associated with water quality and hydrodynamic parameters, as well as to identify likely associations between Odonata fauna and environmental variations in the region.

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